Title

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Journal

Diabetes, 64(12)

ISSN

0012-1797

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Publication Date

2015-12-01

DOI

10.2337/db15-0254

Peer reviewed

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Genetic Evidence for a Causal Role of Obesity in Diabetic Kidney Disease

Diabetes 2015;64:4238–4246 | DOI: 10.2337/db15-0254

Obesity has been posited as an independent risk factor for diabetic kidney disease (DKD), but establishing causality from observational data is problematic. We aimed to test whether obesity is causally related to DKD using Mendelian randomization, which exploits the random assortment of genes during meiosis. In 6,049 subjects with type 1 diabetes, we used a weighted genetic risk score (GRS) comprised of 32 validated BMI loci as an instrument to test the relationship of BMI with macroalbuminuria, end-stage renal disease (ESRD), or DKD defined as presence of macroalbuminuria or ESRD. We compared these results with cross-sectional and longitudinal observational associations. Longitudinal analysis demonstrated a U-shaped relationship of BMI with development of macroalbuminuria, ESRD, or DKD over time. Cross-sectional observational analysis showed no association with overall DKD, higher odds of macroalbuminuria (for every 1 kg/m^2 higher BMI, odds ratio [OR] 1.05, 95% CI 1.03–1.07, $P < 0.001$), and lower odds of ESRD (OR 0.95, 95% CI 0.93–0.97, $P < 0.001$). Mendelian randomization analysis showed a 1 kg/m^2 higher BMI conferring an increased risk in macroalbuminuria (OR 1.28, 95% CI 1.11–1.45, $P = 0.001$), ESRD (OR 1.43, 95% CI 1.20–1.72, $P < 0.001$), and DKD (OR 1.33, 95% CI 1.17–1.51, $P < 0.001$). Our results provide genetic evidence for a causal link between obesity and DKD in type 1 diabetes. As obesity prevalence rises, this finding predicts an increase in DKD prevalence unless intervention should occur.

Diabetic kidney disease (DKD) is a devastating complication of diabetes and is the major cause of end-stage renal disease (ESRD) in the U.S., where the incidence of ESRD has nearly doubled over the past two decades (1). Although tight glycemic control in type 1 diabetes can reduce the rates of DKD and ESRD (2,3), a substantial number of patients develop DKD despite adequate glycemic control, while others with chronic severe hyperglycemia are relatively spared (4). Obesity has been posited as an independent risk factor for both diabetic (5,6) and nondiabetic (7–11) renal disease. However, epidemiologic studies have produced conflicting results, and establishing causality from observational data is difficult (6,12).

Mendelian randomization has emerged as a novel and powerful approach to assess causality, free from the limitations of traditional observational studies and the operational constraints of randomized controlled trials. Given the principle of random assortment of gene variants during meiosis, Mendelian randomization is considered analogous to a randomized controlled trial, where exposure groups are defined by genotype. Others have exploited the heritable nature of obesity in Mendelian randomization

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Received 23 February 2015 and accepted 19 August 2015.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0254/-/DC1.

J.N.T. and E.H.D. contributed equally to this study.

studies to assess the causality of obesity on a variety of cardiometabolic phenotypes, using genetic variants, either individually or in combination, associated with BMI (13–15). In this study, we use Mendelian randomization to evaluate the causal relationship between obesity and DKD (Fig. 1), comparing and contrasting with cross-sectional and longitudinal observational associations.

RESEARCH DESIGN AND METHODS

Study Design

The study design consisted of two components. First, in a cohort of Finnish participants with type 1 diabetes assessed longitudinally for renal complications, we evaluated the association of obesity at the initial visit with the likelihood of developing DKD over time. Second, using a case-control design, we compared the observational association of BMI and DKD with Mendelian randomization analysis of BMI and DKD, using a weighted genetic risk score of BMI-raising alleles as an instrument.

Study Participants

Three case-control cohorts previously participating in the Genetics of Nephropathy—An International Effort (GENIE) consortium (16) contributed genetic and phenotypic data to this study: the Genetics of Kidneys in Diabetes US Study (US-GoKinD [17]); the All Ireland-Warren 3-Genetics of Kidneys in Diabetes U.K. and Republic of Ireland (UK-ROI [18]) Collection; and the Finnish Diabetic Nephropathy Study (FinnDiane [19]). The GoKinD and UK-ROI studies were both cross-sectional in nature, with risk factors and renal status assessed at one time point. The FinnDiane study is an ongoing longitudinal study with baseline examinations beginning in 1998 and follow-up examinations since 2004. Subject recruitment has previously been described elsewhere (20). In brief, study participants attended a regular visit to their physician, during which they were assessed for both micro- and macrovascular complications. For this particular study, FinnDiane patients with BMI and genotype data available were included. Data were extracted from medical files and hospital discharge registries to complement the information from follow-up examination. The study protocol is in accordance with the Declaration of Helsinki and was approved by the local ethics committee at each study center. Each study participant gave written informed consent prior to participation.

For the cross-sectional analyses, all cases had a diagnosis of type 1 diabetes for at least 10 years and were classified as having macroalbuminuria and ESRD. Normal control subjects were defined as individuals with type 1 diabetes for at least 15 years and no evidence of renal disease (Supplementary Table 1). For the longitudinal analyses in the FinnDiane cohort, we included subjects with type 1 diabetes of any duration and no ESRD at baseline with available follow-up data (Supplementary Fig. 1).

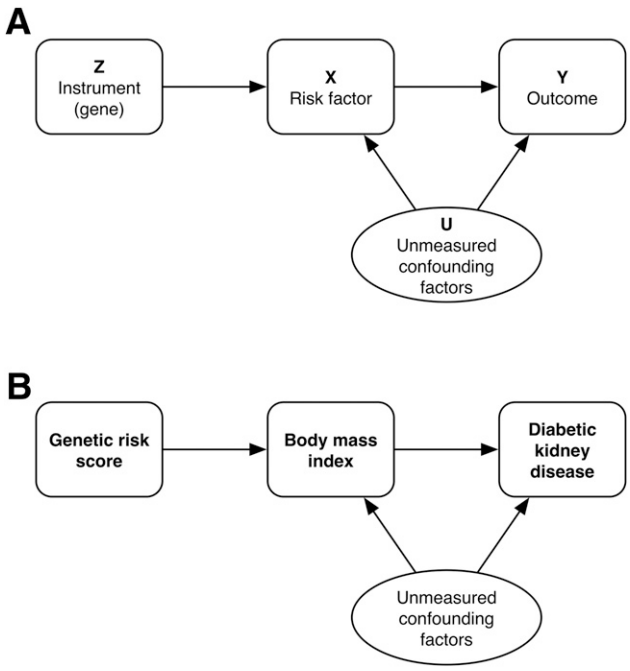

Figure 1—Schematic overview of Mendelian randomization. *A*: Since the observed association between a given risk factor and outcome may be influenced by confounders, a genetic variant that has a direct association with the intermediate risk factor can be used to assess the causal relationship between risk factor and outcome. Note three key assumptions inherent in this depiction: *1*) the variant is independent of the confounders, *2*) the variant is reliably associated with the intermediate risk factor, and *3*) there is no direct effect of the variant on the outcome (i.e., bypassing the risk factor). *B*: In our study, we used a genetic risk score as an instrument for BMI to evaluate the causal relationship of BMI and DKD.

Phenotype Definitions

The phenotype of DKD is now considered to be more complex than the traditional paradigm of steady, inexorable progression from microalbuminuria to macroalbuminuria and ultimately ESRD (16,21–23). Therefore, we studied three definitions of DKD: a broad definition including subjects with either ESRD or macroalbuminuria as case subjects, a stringent definition of case subjects as those with ESRD alone, and case subjects with macroalbuminuria alone. BMI was calculated as weight in kilograms divided by the square of height in meters.

In the longitudinal analyses, we studied *1*) progression from normal albumin excretion rate (AER) or microalbuminuria to broadly defined DKD (macroalbuminuria or ESRD), *2*) progression from normal AER or microalbuminuria to macroalbuminuria, and *3*) progression from normal AER, microalbuminuria, or macroalbuminuria to ESRD. In addition, we studied "any progression," with any new renal event defined as the development of microalbuminuria, macroalbuminuria, or ESRD during follow-up.

Genetic Instruments

Statistical power for Mendelian randomization can be improved by use of multiple genetic variants combined to

give a higher predictive value (24). Therefore, we chose to use a weighted genetic risk score as an instrument for BMI. We calculated our weighted genetic risk score using the 32 lead single nucleotide polymorphisms (SNPs) reported in the largest European genome-wide association study of obesity published by Speliotes et al. (25) at the time of study design. The score was calculated by summing up the number of risk alleles at each locus, multiplied by the effect size at each locus as reported by Speliotes et al., using the PLINK "–score" routine (26). Genotyping and imputation were performed as previously reported (16). GenGen (http://www.openbioinformatics.org) was used to convert the imputed dosage data to most likely genotypes using a threshold of 90% for the genotypic posterior probability. When direct or imputed genotype data were not available at a given locus, the expected value was imputed based on the cohort allele frequency. In the UK-ROI cohort, all subjects had genotype data for at least 30 of the 32 loci. In the FinnDiane cohort, all subjects had genotype data for 28 of 31 loci. (One locus had a high rate of missingness and was excluded for all subjects.) In the US-GoKinD cohort, 1,408 (80%) of subjects had information for at least 29 of 32 loci; analyses were run with and without subjects having information at <29 loci and were not significantly different.

Statistical Analysis

Analyses were performed using either Stata, version 12.1 (StataCorp, College Station, TX), or R (http://www.r-project.org).

Longitudinal Analysis

Using longitudinal data from the FinnDiane study, we assessed the effect of obesity on incident DKD by Cox proportional hazards model with BMI predicting time to DKD progression, adjusted for baseline age, sex, and diabetes duration. BMI was studied both as a continuous and categorical variable, divided into quintiles, with the second quintile used as a reference. In addition, the linearity assumption was tested by fitting a model where BMI was included as a restricted cubic spline with three knots located at the 10th, 50th, and 90th percentiles of BMI (corresponding to 20.99 kg/m^2, 24.84 kg/m^2, and 29.97 kg/m^2). The number and location of knots used to fix splines in the modeling followed previous recommendations (27). The reference value for hazard ratios in the restricted cubic spline model was set to median BMI (24.84 kg/m^2), and Wald tests for linearity were used for testing nonlinear effects.

Association Analysis

Using cross-sectional data from all cohorts, we examined the association of BMI with the genetic risk score using linear regression. The observational estimates of odds ratios (ORs) of each outcome per 1 kg/m^2 higher BMI, adjusted for age, sex, and diabetes duration, were assessed using logistic regression.

Mendelian Randomization

Instrumental variable analysis was used to estimate the causal effect per 1 kg/m^2 higher BMI on DKD outcome, using the logistic control function estimator method (28). In this two-stage method, we first performed a linear regression of BMI on the weighted genetic risk score. The predicted BMI from stage 1 was then used as the independent predictor for DKD outcome in the stage 2 logistic regression, adjusting for the residuals from stage 1. The second stage regression was adjusted for age, sex, and diabetes duration. To assess whether our results were influenced by pleiotropy, we performed a sensitivity analysis using two subsets of the original genetic risk score: one excluding SNPs with nominally significant effects on other metabolic traits (HDL and LDL cholesterol, triglycerides, fasting glucose, fasting insulin, HOMA of insulin resistance, HOMA of β-cell function, 2-h glucose, and type 2 diabetes risk) and one including only SNPs found within genes associated with monogenic obesity syndromes (*MC4R*, *POMC*, *BDNF*, and *SH2B1*). Finally, we performed a statistical comparison of observational and Mendelian randomization analyses using the method of Altman and Bland (29).

RESULTS

Baseline characteristics of FinnDiane longitudinal participants are shown in Table 1 and of the three cohorts participating in the cross-sectional analysis in Table 2.

Table 1—Baseline characteristics of participants in the longitudinal FinnDiane cohort by DKD status

	Normal AER	Microalbuminuria	Macroalbuminuria	ESRD
N	1,538	447	593	319
BMI (kg/m^2)	25.1 ± 3.3	25.7 ± 3.6	26.0 ± 4.0	24.1 ± 3.8
Women (%)	56.9	40.8	40.3	40.4
Age (years)	40.4 ± 12.0	39.3 ± 12.0	41.7 ± 10.4	45.6 ± 8.5
Duration of diabetes (years)	24.4 ± 9.9	25.9 ± 10.5	28.5 ± 8.0	33.0 ± 8.14
HbA_{1c} (%)	8.2 ± 1.3	8.8 ± 1.5	9.1 ± 1.6	8.6 ± 1.5
HbA_{1c} (mmol/mol)	66 ± 14.2	73 ± 16.4	76 ± 17.5	70 ± 16.4

Data are means ± SD unless otherwise indicated. *N*, number of participants.

Table 2—Baseline characteristics of participants in the cross-sectional US-GoKinD, UK-ROI, and FinnDiane cohorts by DKD status

	US-GoKinD		UK-ROI		FinnDiane	
	No DKD	DKD	No DKD	DKD	No DKD	DKD
N	807	761	831	674	1,262	912
BMI (kg/m^2)	26.1 ± 8.6	25.7 ± 5.2	26.2 ± 4.1	26.3 ± 4.7	25.1 ± 3.33	25.3 ± 4.0
Women (%)	58.4	48.2	55.7	40.4	57.5	40.8
Age (years)	38.5 ± 8.6	43.2 ± 6.9	41.6 ± 11.0	48.4 ± 10.6	42.4 ± 11.6	43.1 ± 10.0
Duration of diabetes (years)	25.5 ± 7.7	31.3 ± 7.8	27.1 ± 8.6	33.5 ± 9.5	27.1 ± 9.0	30.0 ± 8.3
HbA_{1c} (%)	7.5 ± 1.2	7.5 ± 1.9	8.6 ± 1.6	8.9 ± 1.8	8.2 ± 1.3	8.9 ± 1.6
HbA_{1c} (mmol/mol)	58 ± 13.1	58 ± 20.8	70 ± 17.5	74 ± 19.7	66 ± 14.2	74 ± 17.5
ESRD (%)	0	65.4	0	33.1	0	35.0

Values are means ± SD except where indicated. *N*, number of participants.

For the longitudinal analysis, 2,392 participants (1,386 normoalbuminuric, 401 microalbuminuric, and 605 macroalbuminuric) were followed for a median duration of 6.5 years. In the cross-sectional analysis, there were 1,040 participants with ESRD, 1,307 participants with macroalbuminuria, and 2,900 control subjects without any clinical evidence of kidney disease.

We first examined the longitudinal FinnDiane cohort to test the observational relationship of BMI with time to DKD, using the selected end points as outlined above. At the follow-up visit, renal disease progression had occurred for 17.6% (N = 420). There was no significant linear relationship of BMI with any of the DKD outcomes in the Cox proportional hazards model. Analysis by BMI quintiles demonstrated a U-shaped relationship between obesity and progression of DKD, with higher risk in the lowest and highest quintiles, for all outcomes (Table 3; Fig. 2). In addition, we obtained significant P values for nonlinear effects in the restricted cubic spline model supporting the existence of a nonlinear, U-shaped relationship for BMI and progression to DKD (P = 0.018) as well as progression to macroalbuminuria (P = 0.019) and ESRD ($P < 0.001$) (Fig. 2).

Using our cross-sectional data from all three cohorts, we examined the association of BMI with presence of any of the three DKD definitions outlined above (Fig. 3, left panel). There was no significant association of BMI with broadly defined DKD in any of the three cohorts (combined OR for per 1 kg/m^2 higher BMI 1.00, 95% CI 0.99–1.02, P = 0.62). Higher BMI was associated with increased odds of macroalbuminuria alone (OR 1.05, 95% CI 1.03–1.07, $P < 0.001$). For ESRD alone, the odds were lower for each 1 kg/m^2 higher BMI (OR 0.95, 95% CI 0.93–0.97, $P < 0.001$).

We proceeded to perform a Mendelian randomization analysis, using genetic variants associated with elevated BMI, to assess the causal effect of higher BMI over the life span on development of DKD. First, to validate the association of the selected BMI-raising alleles in our cohorts, we performed a linear regression of BMI on the weighted genetic risk score. Meta-analysis of the three studies showed that BMI was higher by 0.42 kg/m^2 per 1 SD of the weighted genetic risk score (95% CI 0.32–0.52, $P < 0.001$). The results were similar across all participants, regardless of proteinuria or ESRD status.

Next, we used the weighted genetic risk score in an instrumental analysis of BMI with each of our defined DKD outcomes. We observed evidence for causality of BMI as determined by genetics with all three DKD outcomes (Fig. 3, right panel). For every 1 kg/m^2 higher BMI, meta-analysis showed an overall increased odds of broadly defined DKD (combined OR 1.33, 95% CI 1.17–1.51, $P < 0.001$), macroalbuminuria alone (OR 1.28, 95% CI 1.11–1.45, P = 0.001), and ESRD alone (OR 1.43, 95% CI 1.20–1.72, $P < 0.001$) in the combined total of all three cohorts. Results did not change significantly after adjustment for population stratification with principal components. Sensitivity analyses using subsets of the original genetic risk score to minimize pleiotropy showed a direction of effect consistent with our original findings for both subsets (data not shown).

In summary, cross-sectional observational analysis of the association of BMI with DKD showed no increased risk of ESRD or the combined phenotype of macroalbuminuria plus ESRD but a 5% increased risk of macroalbuminuria for every 1 kg/m^2 higher BMI. In a longitudinal observational analysis, there appeared to be a U-shaped relationship of BMI with development of DKD over time, with increased risk for the lowest and highest quintiles of BMI. With Mendelian randomization analysis, there appeared to be a causal association with BMI as determined by genetics and any of the three DKD outcomes, with a 1 kg/m^2 higher BMI conferring a 28% increased risk in macroalbuminuria alone, a 43% increased risk in ESRD alone, and a 33% increased risk of DKD defined as either macroalbuminuria or ESRD.

DISCUSSION

In this study, we used Mendelian randomization to assess causality of obesity on DKD and compared our

Table 3—Longitudinal association of BMI with DKD outcomes

Quintile (BMI range), kg/m^2	Macro or ESRD	*N*	Macro alone	*N*	ESRD alone	*N*	Any progression	*N*
1 (14.99–22.28)	**2.05 (1.09–3.86)**, 0.026	309	**2.33 (1.19–4.58)**, 0.014	309	**2.04 (1.36–3.07)**, 5.5×10^{-4}	394	**1.92 (1.39–2.65)**, 7.2×10^{-5}	427
2 (22.28–24.03)	1.00	379	1.00	376	1.00	454	1.00	473
3 (24.03–25.71)	0.92 (0.45–1.90), 0.82	349	0.94 (0.42–2.07), 0.87	347	1.31 (0.86–1.98), 0.21	460	1.21 (0.87–1.70), 0.26	479
4 (25.72–27.99)	1.48 (0.79–2.78), 0.23	348	1.56 (0.79–3.10), $P = 0.20$	345	0.73 (0.46–1.17), 0.19	445	1.08 (0.77–1.51), 0.67	485
5 (27.99–52.45)	**1.95 (1.04–3.65)**, 0.038	**309**	**1.58 (1.06–2.36)**, 0.026	307	**1.58 (1.06–2.36)**, 0.026	445	**1.63 (1.19–2.24)**, 0.0024	483

Data are OR (95% CI), *P* value unless otherwise indicated. Boldface type indicates results that are nominally significant ($P < 0.05$). Macro, macroalbuminuria; *N*, number of participants.

results with both cross-sectional and longitudinal observational associations of BMI and DKD. The observational data hint at a relationship of obesity with DKD, but the findings do not lead to a straightforward interpretation. In the cross-sectional analysis, there is a positive association of risk of macroalbuminuria with increasing BMI, but ESRD is associated with lower BMI; this finding may represent reverse causation, as patients with ESRD are ill and often cachectic. The longitudinal analysis shows that subjects at the highest and lowest quintiles of BMI were both at higher risk of being diagnosed with kidney disease at follow-up; it is unclear whether leanness is itself a risk factor for disease or a marker that the disease process is present but has not reached a threshold for diagnosis at first evaluation. In contrast, the Mendelian randomization analysis supports the conclusion of a causal role for obesity in the development of DKD, whether defined by presence of proteinuria or more strictly by progression to ESRD.

The validity of these findings depends on several key assumptions required for Mendelian randomization: *1*) that the genetic instrument is independent of any confounders, *2*) that the genetic instrument is reliably associated with the intermediate phenotype, and *3*) that the genetic instrument is independent of the outcome; i.e., the genetic variant does not have a direct effect on the outcome other than through the intermediate phenotype (30). Both the first and third assumptions are subject to violation from population stratification and pleiotropic effects (either directly or from genes in linkage disequilibrium with the variant used). In this study, inclusion of principal components in the analysis did not change the results significantly. The use of multiple variants in a genetic risk score, as used in this study, may help balance out the effect of pleiotropy in the sense of a given individual variant affecting multiple phenotypes (31,32), although systematic pleiotropic effects across many loci could still theoretically be present. (Neither this nor any other Mendelian randomization approach rules out indirect causal effects in the form of downstream events; i.e., higher BMI causally leads to increased blood pressure, which in turn increases DKD risk.) When we performed sensitivity analysis using two different subsets of the BMI genetic risk score, our results were directionally consistent for both subsets, suggesting that pleiotropy does not explain our results. With regard to the second assumption, the variants used in our genetic risk score were previously validated in a large, well-constructed genome-wide association study meta-analysis of obesity and were strongly associated with BMI in all three of our cohorts. We used multiple variants and a weighted score to increase precision (24). These variants together only explain 1.45% of the variance in BMI (25), limiting the power of our analysis. We sought to maximize power by combining findings from three separate cohorts; the overall consistency observed between these three

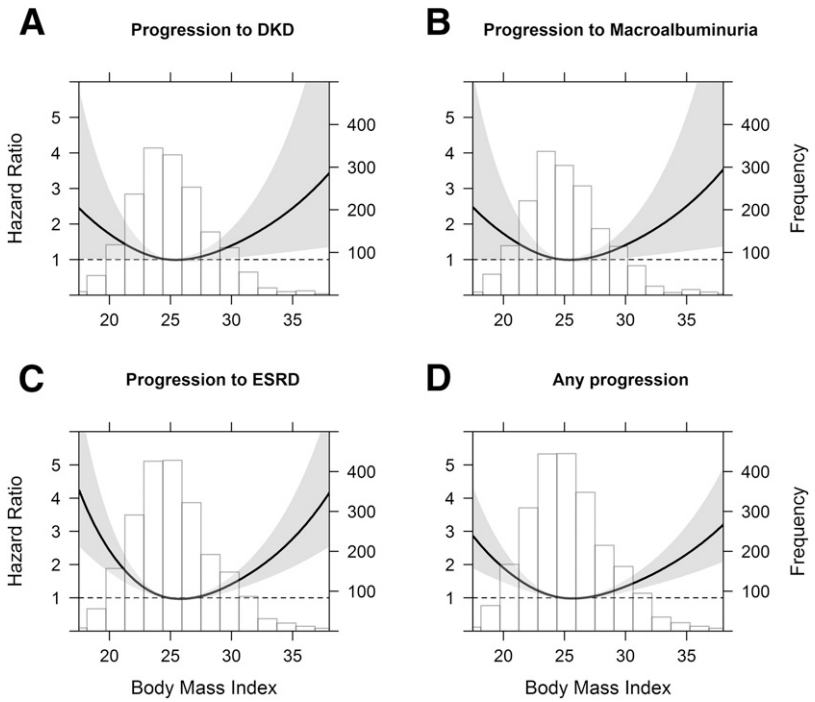

Figure 2—Longitudinal associations between BMI and DKD outcomes, allowing for nonlinear effects, with 95% CI. Results obtained by multivariable Cox regression (left *y*-axis) with restricted cubic splines with three knots for BMI, adjusted for age, sex, and duration of diabetes at baseline, overlaying a histogram displaying the distribution of BMI (right *y*-axis, number of subjects).

independent cohorts lends strength to our findings. Finally, we note that our findings are limited to European populations, as the variants used to construct our genetic risk score were identified in European subjects and the cohorts used in our study are all of European descent.

Our findings support a causal role for obesity in development of DKD but do not explain the underlying mechanism. Obesity has well-known links to dyslipidemia, hypertension, and insulin resistance. Prior studies in patients with type 1 diabetes suggest that some or all of these risk factors are associated with increased risk of complications. In the Diabetes Control and Complications Trial (DCCT), investigators examined whether the presence of metabolic syndrome (defined as central obesity and presence of either raised triglycerides or hypertension) or insulin sensitivity (represented by an estimated glucose disposal rate [eGDR] calculated from the waist-to-hip ratio, hypertension status, and HbA_{1c}) at baseline was associated with increased risk of complications. They found that the most insulin-resistant patients (low eGDR) had the highest risk of microvascular and macrovascular complications; presence of the metabolic syndrome had little predictive value (33). In the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study, both insulin resistance (measured by eGDR) and presence of metabolic syndrome were associated with increased risk of nephropathy, although the strength of the metabolic syndrome association depended on the criteria used (34). The individual components of the metabolic syndrome (elevated triglycerides, hypertension, high waist-to-hip ratio) were all predictive of major outcomes of diabetes (coronary artery disease, renal failure, or death from any diabetes-related cause). In an earlier study of the FinnDiane cohort, the presence of metabolic syndrome or any one of its components (increased waist circumference, hypertension, elevated triglycerides, or low HDL) was associated with increased risk of diabetic nephropathy (20). Future well-powered Mendelian randomization studies estimating the causal effects of triglycerides or hypertension on DKD could help untangle some of these questions.

Understanding the burden of obesity on patients with type 1 diabetes has gained importance as rates of obesity and overweight are on the rise both in the general population (35) and in those with type 1 diabetes (36–39). Weight gain is a known adverse effect of intense glycemic control (2); whether this type of insulin-induced weight gain is influenced by genetic variants commonly associated with BMI remains to be tested. Recent work from the follow-up study of DCCT participants (the Epidemiology of Diabetes Interventions and Complications [EDIC]) has shown an association of excess weight gain with central obesity, dyslipidemia, hypertension, and insulin resistance, as well as more extensive atherosclerosis (40). Given recent trends in obesity rates, our finding predicts a rise in rates of kidney disease in the population with type 1 diabetes. If further research validates our findings, weight management

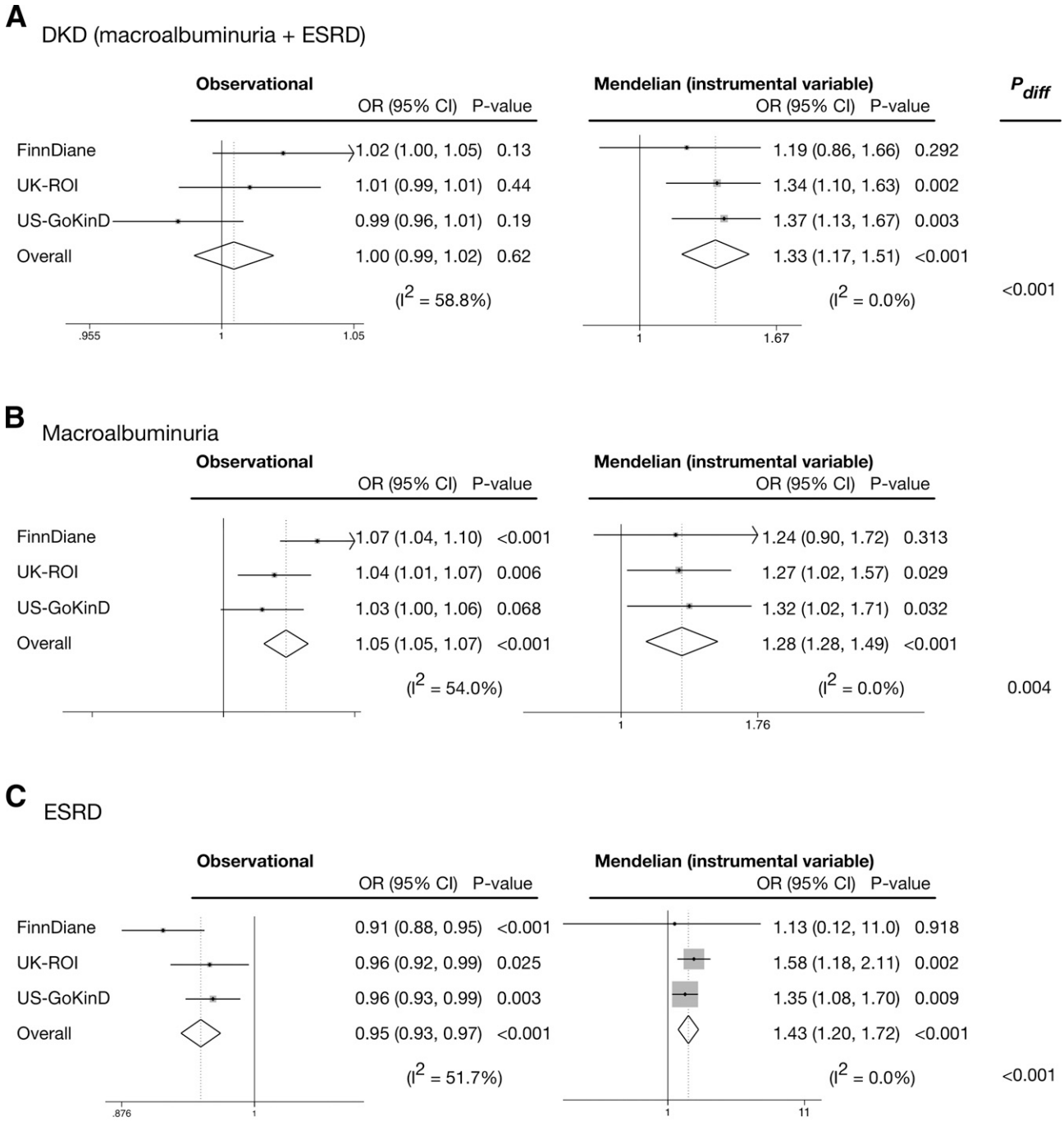

Figure 3—Comparison of observational (left panel) vs. instrumental variable (right panel) analysis of the association of BMI with DKD outcomes in all three cohorts. ORs are reported per 1 kg/m^2 higher BMI. *A*: DKD (macroalbuminuria or ESRD). *B*: Macroalbuminuria alone. *C*: ESRD alone. P_{diff}, *P* value for statistical comparison between observational and instrumental analysis.

may become an important adjunct to glycemic control for reducing complication risk.

Acknowledgments. The authors thank all the FinnDiane physicians and nurses at each participating center for patient recruitment and the collection of samples and data (Supplementary Table 3).

Funding. J.N.T. was supported by National Institutes of Health Training grant T32-DK-007260 and grant F32-DK-103486-01. E.H.D. was supported by grants from the Nylands Nation. R.M.S was supported by JDRF grants 3-APF-2014-111-A-N and 3-2011-70. N.S. was supported by grants from the Waldemar von Frenckell Foundation. The FinnDiane Study Group was supported by grants from the Folkhälsan Research Foundation, Liv och Hälsa Foundation, the Willhelm and Else Stockmann Foundation, Helsinki University Central Hospital Research Funds, the Sigrid Juselius Foundation, the Finnish Cultural Foundation, the

Signe and Ane Gyllenberg Foundation, Finska Läkaresällskapet, Academy of Finland (134379), Novo Nordisk Foundation, and Tekes. A.J.M., A.P.M., D.S., and C.G. are supported by the US-Ireland R&D partnership. The Warren 3/U.K. GoKinD Study Group was jointly funded by Diabetes UK and JDRF and includes: Belfast: A.P. Maxwell, A.J. McKnight, D.A. Savage; Edinburgh: J. Walker; London: S. Thomas, G.C. Viberti; Manchester: A.J.M. Boulton; Newcastle: S. Marshall; Plymouth: A.G. Demaine, B.A. Millward; Swansea: S.C. Bain. This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases grant R01-DK-081923 to P.-H.G., J.N.H., and J.C.F.

Duality of Interest. P.-H.G. has received research grants from Eli Lilly and Roche; is an advisory board member for Abbott, Abbvie, Boehringer Ingelheim, Cebix, Eli Lilly, Janssen, Medscape, and Novartis; and has received lecture fees from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Genzyme, Novartis, Novo Nordisk, and Merck Sharp & Dohme. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. J.N.T. and E.H.D. designed the study and wrote the manuscript. J.N.T. performed the analysis in the US-GoKinD and UK-ROI cohorts with input from R.M.S., J.N.H., and J.C.F. E.H.D. performed all analyses in the FinnDiane cohort with input from N.S., C.F., and P.-H.G. R.M.S., N.S., C.F., E.B., C.G., P.-H.G., J.N.H., and J.C.F. reviewed and commented on the manuscript. All authors reviewed the manuscript, agree with the manuscript results and conclusions, and approved the final draft. P.-H.G. and J.C.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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